



저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:



저작자표시. 귀하는 원저작자를 표시하여야 합니다.



비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.



변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 [이용허락규약\(Legal Code\)](#)을 이해하기 쉽게 요약한 것입니다.

[Disclaimer](#)

Nucleic Acid Extraction Method Using SiN_x Nanofilter Device

나노 필터 디바이스를 이용한 핵산 추출 방법에
관한 연구

2018 년 2 월

서울대학교 대학원
재료공학부
이 다 은

Nucleic Acid Extraction Method Using SiN_x Nanofilter Device

지도 교수 김 기 범

이 논문을 공학석사 학위논문으로 제출함

2018 년 2 월

서울대학교 대학원

재료공학부

이 다 은

이다은의 공학석사 학위논문을 인준함

2018 년 2 월

위 원 장 _____ 남기태 (인)

부위원장 _____ 김기범 (인)

위 원 _____ 선정윤 (인)

Nucleic Acid Extraction Method Using SiN_x Nanofilter Device

A DISSERTATION SUBMITTED TO
DEPARTMENT OF MATERIALS SCIENCE
AND ENGINEERING
SEOUL NATIONAL UNIVERSITY

FOR THE DEGREE OF
MASTER

Da Eun Lee
February 2018

Abstract

Current diagnosis of diseases is an important issue in the biological field. The diagnosis of the disease consists of four stages. Sampling, genetic material separation, amplification and analysis for genetic material. In this study, we tried to isolate the genetic material with a new idea. Since DNA has negative charge, it would move in the electric field. In this basic principle, in one direction electric field, genetic material would be collected in a chamber. However, in the electric field, all charged materials would move but because of high charge-to-mass ratio in DNA, it would transfer fast and firstly. The advantage of this system is that it is a fast, one-step process and minimizes damage to the DNA while minimizing the use of chemical solutions.

In this study, we developed a filter membrane to embody this idea. This filter membrane has millions of pores of 50-200 nm in size, which do not interfere with DNA migration and interfere with other cell debris and proteins. This study was carried out from the chamber design because there was no preceding study. The design of the chamber was confirmed by simulating the pattern of the electric field. Later, to better understand the trends according to the experimental variables, the experimental results were predicted and compared using the model. However, there was a difference between the experimental data and the numerical modeling values because of limits of the numerical model.

Keywords: nanofilter, electric field, DNA translocation, chamber design

Student number: 2016-20807

TABLE OF CONTENTS

Abstract	i
Table of contents.....	ii
List of figures	iv
Chapter 1. Introduction.....	1
1-1 Introduction of DNA extraction	2
1-2 Concept of nanofilter system.....	6
1-3 Outline of the dissertation	8
1-4 References	9
Chapter 2. Nanofilter Fabrication.....	10
2-1 Fabrication process	11
2-2 Fabrication results.....	14

Chapter 3. Chamber Design 18**3-1 Introduction 19****3-2 COMSOL Multiphysics Simulation 19****3-3 Results..... 24****Chapter 4. Numerical Modeling of DNA
Translocation and Experimental Results 27****4-1 Introduction 28****4-2 Numerical modeling 28****4-3 Experimental results 32****4-4 Discussion 45****Chapter 5. Summary and Conclusion..... 47****Abstract (In Korean)..... 49****Acknowledgement (In Korean)..... 50**

LIST OF FIGURES

Chapter 1. Introduction

Figure 1-1 Diagnosis process.....	4
Figure 1-2 Commercial kits	4
Figure 1-3 Silica membrane based DNA extraction system....	5
Figure 1-4 Nanofilter system.....	7

Chapter 2. Nanofilter Fabrication

Figure 2-1 Fabrication procedure	12
Figure 2-2 Membrane SEM analysis	13
Figure 2-3 Picture of nanofilter	15
Figure 2-4 Atomic layer deposition results.....	16
Figure 2-5 EDS analysis for Al ₂ O ₃ deposited membrane	17

Chapter 3. Chamber Design

Figure 3-1 One chamber system picture.....	21
Figure 3-2 Geometry of system.....	22
Figure 3-3 Boundary conditions in chamber	22
Figure 3-4 Calculation of mesh.....	23
Figure 3-5 Modeling results	23
Figure 3-6 Two chamber system picture.....	25
Figure 3-7 COMSOL multiphysics simulation result.	26

Chapter 4. Numerical Modeling of DNA Translocation and Experimental Results

Figure 4-1 Experimental results.....	33
Figure 4-2 Experimental results by time	35
Figure 4-3 Experimental results by voltage	37
Figure 4-4 Experimental results by concentration	39
Figure 4-5 Experimental results by pore size.....	41
Figure 4-6 Experimental results by variables	43
Figure 4-7 Comparison with experimental values and numerical modeling values	44
Figure 4-8 The concept of assumption.....	46

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]

Chapter 1. Introduction

1. Introduction of DNA extraction

In current biotechnology studies, rapid and accurate diagnosis of disease is an important factor. In order to diagnose the disease quickly and accurately, a polymerase chain reaction (PCR) test should be performed. At this time, it is very important to extract only DNA from cell debris as a pretreatment process for PCR. [Figure 1-1]

These procedures are the process of taking a sample and analyzing the presence or amount of a specific DNA in the sample. First collect the samples. After that, only the DNA is extracted, and the resultant is amplified by PCR, and the results is analyzed, so that the presence or amount can be known accurately.

In this process, DNA extraction is one of the important steps before performing the subsequent PCR. PCR solution should contain only DNA, which is a genetic material, and no other protein or cell debris.

In these days, DNA extraction methods currently used are mainly commercial kits.[1] [Figure 1-2] Three representative kits are shown below. Silica membrane based DNA extraction, magnetic bead based DNA extraction, and ion exchange based DNA extraction. All three methods use a charge nature to bind DNA with a negative charge to a substance with a positive charge. [Figure 1-3] For example, silica-based DNA extraction uses charge nature.[2] Once the sample is ready, the contents in cell should be came out. This is called lysis process. Lysis refers to the process of breaking the cell walls and causing the genetic material to come out of the cell. In lysis step, sample should be heated by hot plate. Also, a mixture of Gu-HCl, EDTA, Triton X-100

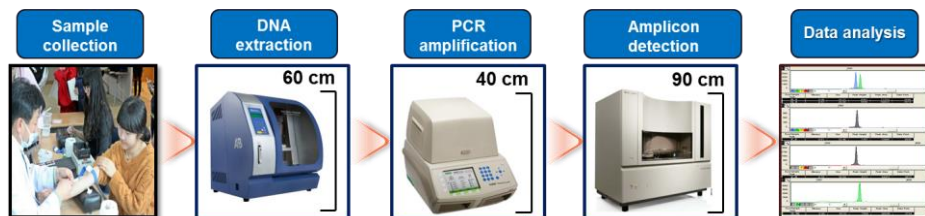
and IPA, known as detergent is added to break cell wall. Then load the lysis solution into the column. At this time, chaotropic agents are added for surface treatment.[3] The chaotropic salts create a hydrophobic environment. Under these hydrophobic conditions, the silica membrane of the columns is the most suitable binding partner for the nucleic acids. Proteins, metabolites, and other contaminants do not bind to the membrane and therefore are washed away during the subsequent washing steps. This washing process can be done with a centrifuge, and should be done several times. After washing process, DNA bound to the silica membrane would be obtained by elution. As written, commercial kit requires several steps such as lysis, DNA binding, multiple washing steps, and elution step. And not only the complex process but also the addition of several chemicals is a disadvantage of commercial kits. Another critical issue is that a certain amount of DNA is always lost in the washing and binding process. For this reason, very high concentration of cell concentration is required.[4] (10^9 cells/ml)

So we eager to develop a preprocessing process in an easy and simple way using Nanofilter. Based on nano technology, we tried to establish detection and early diagnosis system.

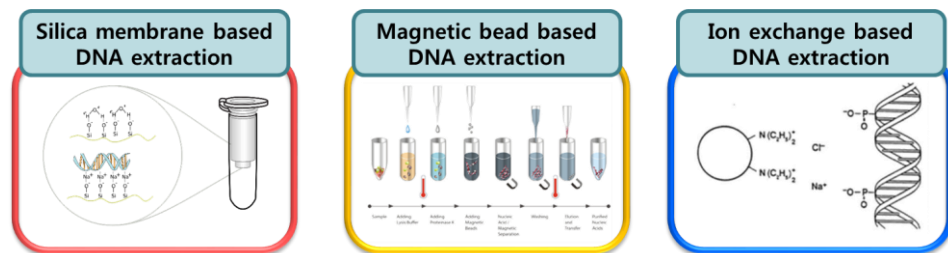
[텍스트 입력]

[텍스트 입력]

[텍스트 입력]



[Figure 1-1] Diagnosis process

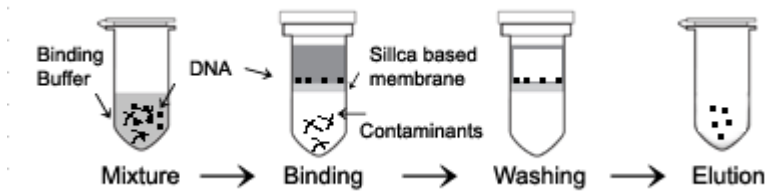


[Figure 1-2] Commercial kits

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]



[Figure 1-3] Silica membrane based DNA extraction system

2. Concept of nanofilter system

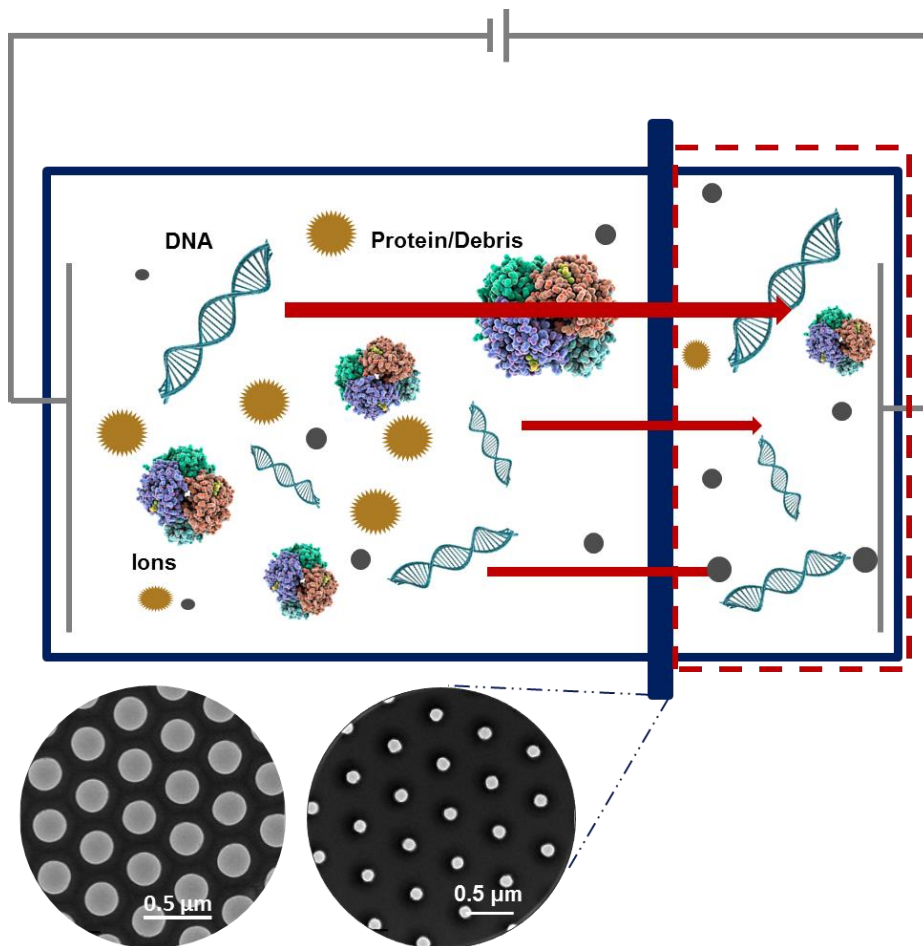
DNA extraction process was briefly reviewed. Currently, common extraction methods have complex steps and each step contains various additives. These are weak points in conventional extraction methods. To make extraction methods simple and intact process, we suggest new concept. We developed a fast and intact DNA extraction method using the fact that charged particle would move in electric field area. It is key concept that DNA would move to collector chamber in electric field area because DNA has negative charge. To extract pure DNA from lysis solution, cell debris and protein should not transfer to collector chamber. We thought up filter to prevent translocation of cell debris and protein.

To materialize the concept, reservoir and collector chamber was prepared and between two chambers, filter was placed. [Figure 1-4] Place lysis solution containing protein and cell debris in a reservoir. Electric field was applied in chamber to transfer DNA to collector which is opposite chamber of reservoir. Highly charged materials would migrate to the collector fast and firstly. DNA is known as high charge to mass ratio material so that DNA would migrate to collector chamber fast and firstly.[5] And also a special kind of protein (those with large charge to mass ratios) would transfer to collector. And inserted nanofilter would prevent protein and cell debris to transfer to collector chamber. With nanofilter system, the goal of this research is to transfer certain amount of DNA and to reduce the amount of protein. As with this system, collector solution can be used immediately for PCR test.[6]

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]



[Figure 1-2] Nanofilter system

3. Outline of the dissertation

We introduced DNA extraction method and proposed to extract DNA with new idea. Nanofilter should be fabricated to implement idea. In the 1cm* 1cm size nanofilter, 1mm * 1mm size membrane is fabricated. And in it, several millions of pores are arrayed. DNA movement should be confirmed through nanofilter. To establish nanofilter system, chamber should be designed. In this thesis, chamber design is very important. The most important thing in chamber design is that the electric field should be evenly distributed in the chamber. To verify this, we used the COMSOL Multiphysics Simulation program. We would discuss about experiments after completing membrane fabrication and chamber fabrication. Before experimentation, numerical modeling was performed to predict DNA translocation. The experiment will be done according to voltage, time, DNA solution concentration, membrane pore size. The experimental results and modeling will be compared and analyzed.

4. References

- [1] Siun Chee Tan, *J Biomed Biotechnol* **10**, 1155 (2009)
- [2]<http://www.komabiotech.co.kr/www/product/productdesc.phtml?seq=254> (25 Dec, 2017)
- [3] Kelly M. Elkins, Towson University, *ELSEVIER* **10**, 1016 (2010)
- [4] Androniki Psifidi, Georgios Banos, *PLoS One* **10**, 1371 (2015)
- [5] Pei Yun Lee, Yong Hoon Kim, *J Vis Exp.* **62**, 3923 (2012)
- [6] John M. Butler, National Institute of Standard and Technology, *ELSEVIER* **11**, 1016 (2011)

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]

Chapter 2. Nanofilter Fabrication

1. Fabrication process

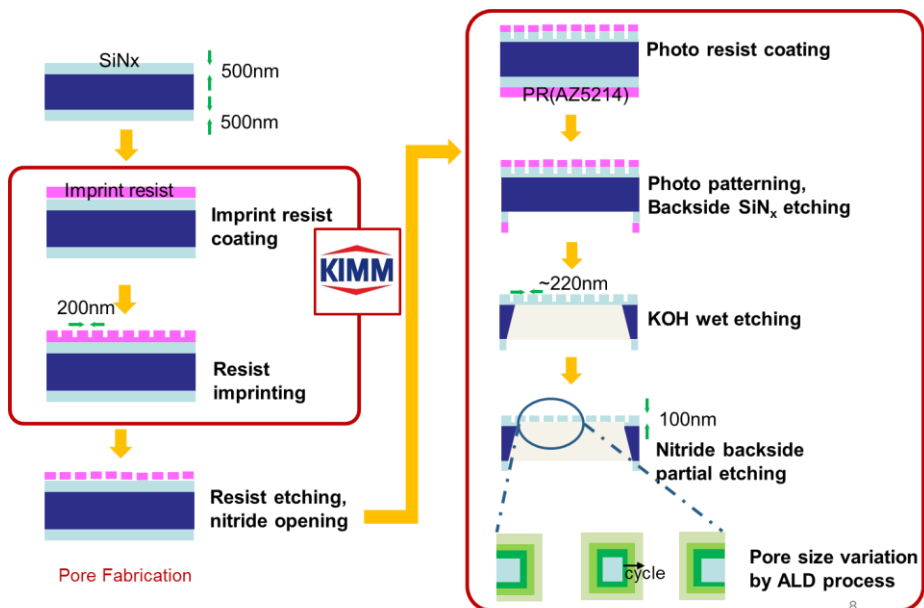
To fabricate Nanofilter membrane, semiconductor processes such as nanoimprint, dry etching, and wet etching are used. The figure below this part shows whole fabrication procedure. [Figure 2-1]

500 nm silicon nitride is deposited on a double-sided polished silicon substrate by LPCVD method. A circular imprint pattern with a diameter of 200 nm and a spacing of 400 nm is prepared by E-beam lithography. Silicon nitride was etched for 400nm using dry etching method. The backside is patterned with 1mm x 1mm by photolithography, and then silicon nitride is dry-etched so that the silicon is exposed. To utilize the anisotropic etching characteristics of single crystal silicon by KOH - IPA mixed solution, the entire substrate was immersed in a potassium hydroxide (KOH) solution at 84 ° C to form a silicon nitride membrane. The back side was etched with RIE system to 100nm thickness to expose silicon nitride membrane. And the nanopore structure is revealed and the nanofilter membrane is completed. In this study, it was possible to fabricate a membrane with 1 mm width, 100 nm thickness, and about 5,000,000 nanopore structures. [Figure 2-2]

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]

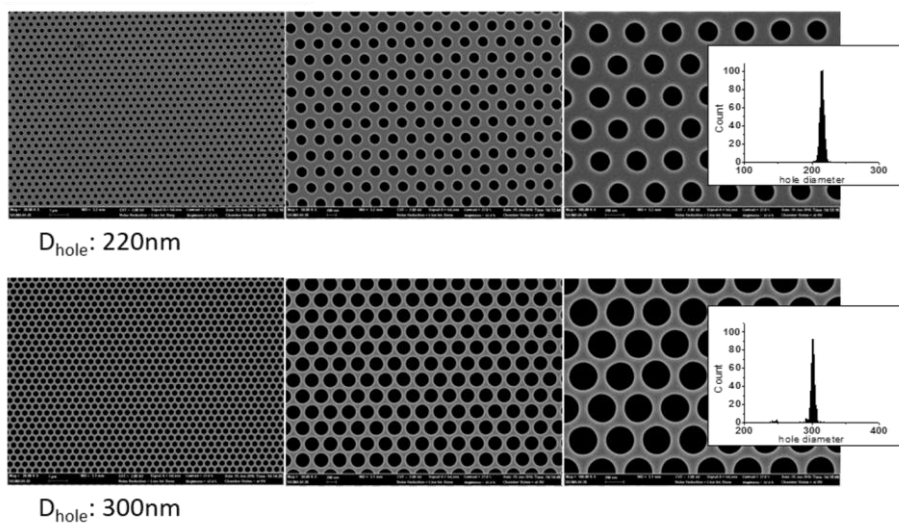


[Figure 2-1] Fabrication procedure

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]



[Figure 2-2] Membrane SEM analysis

2. Fabrication results

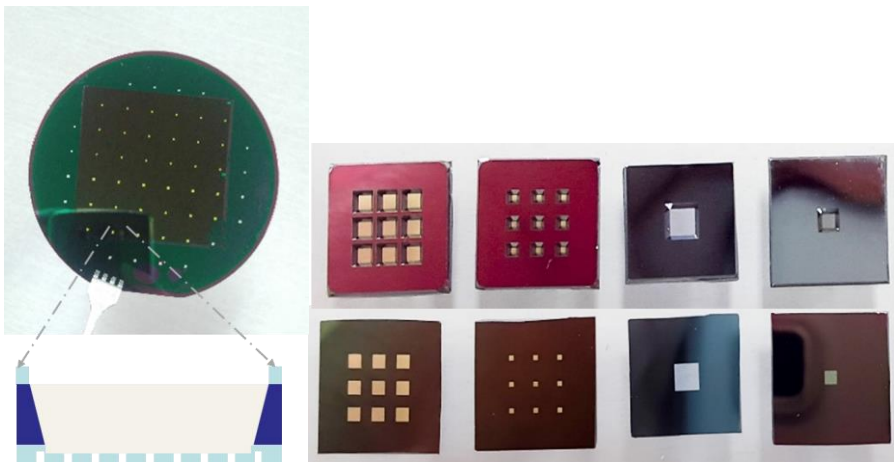
Nanofilter devices were fabricated using nanopore fabrication process. The nanofilter is consisting of size of diameter about 220 nm or 300 nm, a spacing of 400 nm, and a thickness of about 100 nm. (Figure: SEM image and size distribution of nanopore structures with average 220 nm (top) or 300 nm (bottom) size) The diameter of the nano-pores can be controlled by dry etch time during the process. Respectively, the uniformity of the structure was confirmed to be maintained in the area of mm^2 . It is confirmed that about 5,000,000 nano pores per device with a size of 1 mm width. Furthermore, Nanofiler fabrication system was established in wafer size process. [Figure 2-3]

Due to the resolution of the electron beam lithography mask, in the nanofilter, 200nm pore is the largest nano pore size. To reduce pore size, atomic layer deposition (ALD) methods was used. The ALD process is considered to be a very effective way to reduce pore size while maintaining the nano-filter structure, because the deposited characteristic – conformal deposition. Among various materials, Al_2O_3 was selected and deposited. Tis methyl aluminum was used for precursor and water was used as reactant. The deposition temperature is 220oC and the growth rate is 0.12 nm / cycle. [Figure 2-4] This can be seen in the graphs and SEM pictures. We were able to reduce pore size to 100 nm from 250 nm pore size. We also performed EDS analysis. The distance is the distance from the SiN substrate to the center of the pore. Initially, in substrate area, silicon and nitride peaks are formed. And peak of silicon and nitride decreases as aluminum and oxygen peaks increase. Based on the EDS analysis, it was confirmed that Al_2O_3 was deposited conformally. [Figure 2-5]

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]

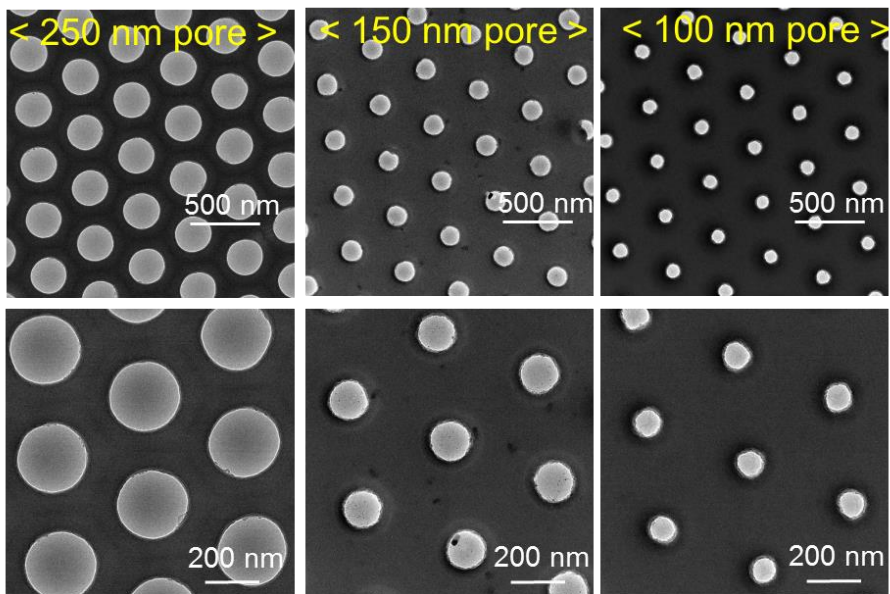
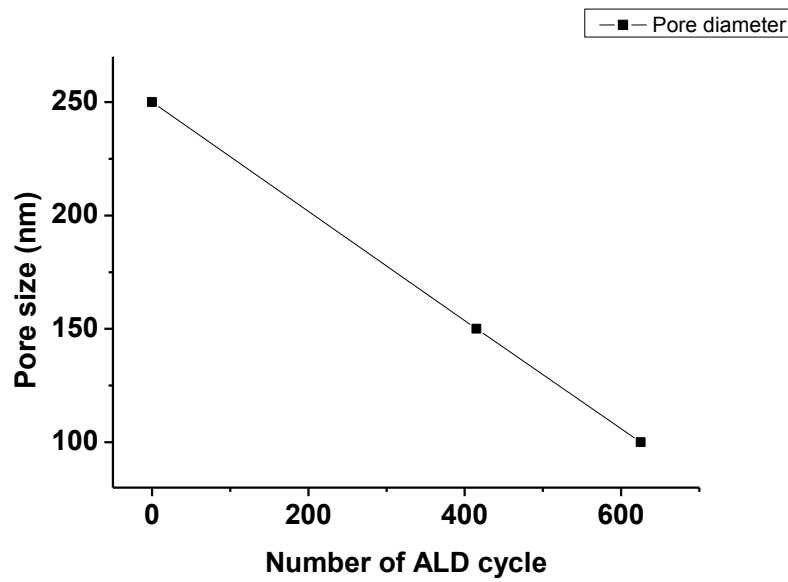


[Figure 2-3] Picture of nanofilter

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]

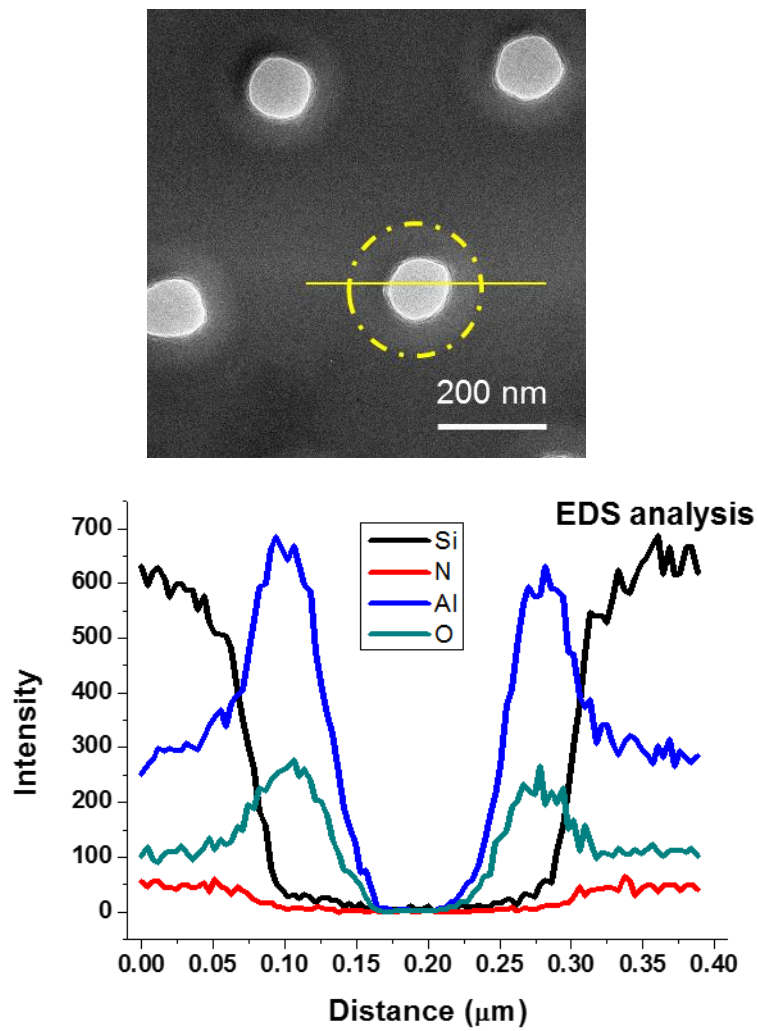


[Figure 2-4] Atomic layer deposition results

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]



[Figure 2-5] EDS analysis for Al_2O_3 deposited membrane

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]

Chapter 3. Chamber Design

1. Introduction

A chamber was prepared for a preliminary experiment. For the preliminary experiment, a rectangular chamber was fabricated. This chamber is divided into a reservoir and a collector, in between two chambers a filter is inserted. The problem with one chamber system was leakage problem. Leakage occurs in which the filter inserted area. [Figure 3-1] So we decided to renew the chamber design. Here are some things to consider for design chamber system. The leakage problem should be solved and a chamber size should be designed considering the volume of DNA extraction solution. The most important thing is that the electric field should be evenly applied to the chamber, and we used the COMSOL Multiphysics Simulation Program to verify electric field.

2. COMSOL Multiphysics Simulation

A commercial Finite Element Method simulation tool (COMSOL multiphysics 4.2. The overall simulation is comprised of four subparts. In the first step, the geometry of each chamber was defined. We used 2D projection of real system. Also millions of pores were neglected but membrane was regarded as a big hole. The thickness of membrane was the geometry values. [Figure 3-2] And in next step, boundary condition and physics in chamber were settled. First, fluid flow in chamber and ionic movement in solution were neglected but electric field. Except electrodes, all surface was settled as zero surface charge. Moreover, reservoir electrode is ground electrode and collector chamber electrode has potential of 4V. In addition, the solution in chamber was considered as water. [Figure 3-3] In third step, mesh was calculated. The smaller

[텍스트 입력]

[텍스트 입력]

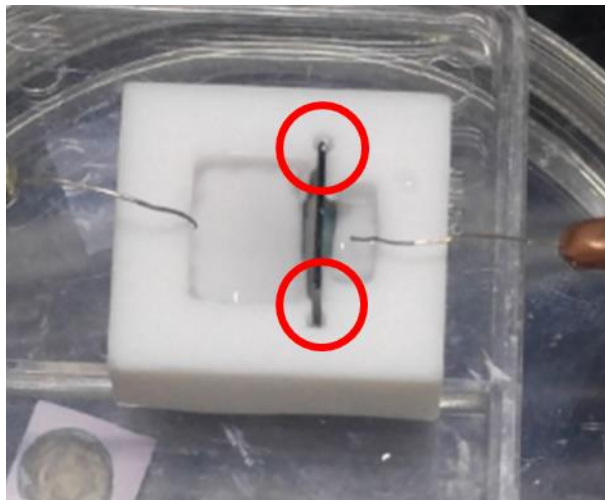
[텍스트 입력]

the mesh size, the finer results came out and more time was needed.
[Figure 3-4] In final step, electric field in chamber is calculated from
the electric potential distribution data. [Figure 3-5]

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]

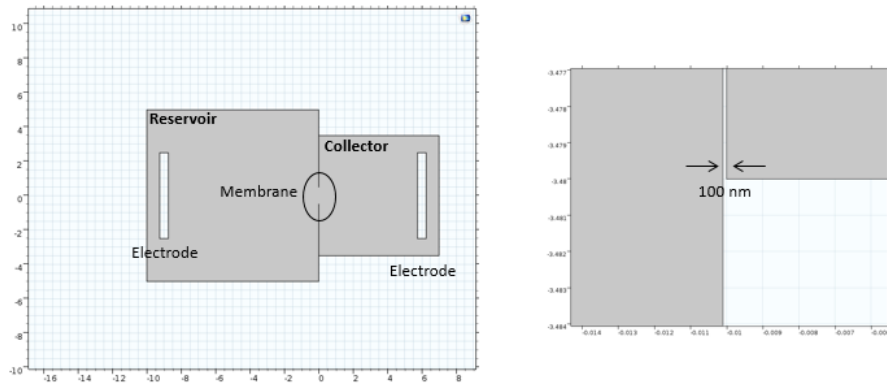


[Figure 3-1] One chamber system picture

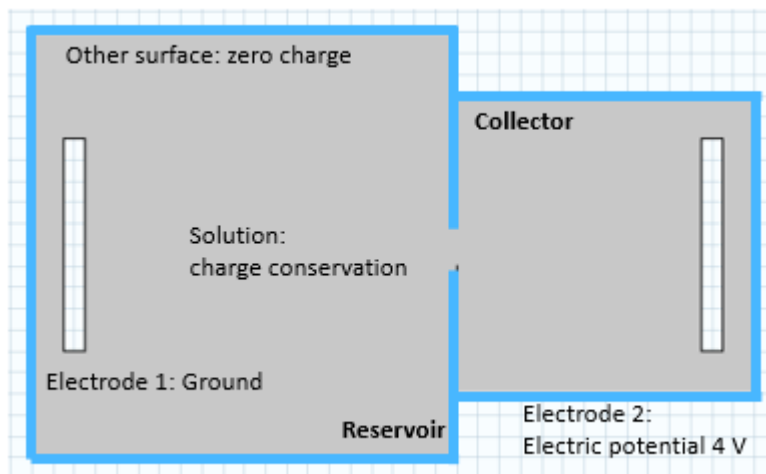
[텍스트 입력]

[텍스트 입력]

[텍스트 입력]



[Figure 3-2] Geometry of system

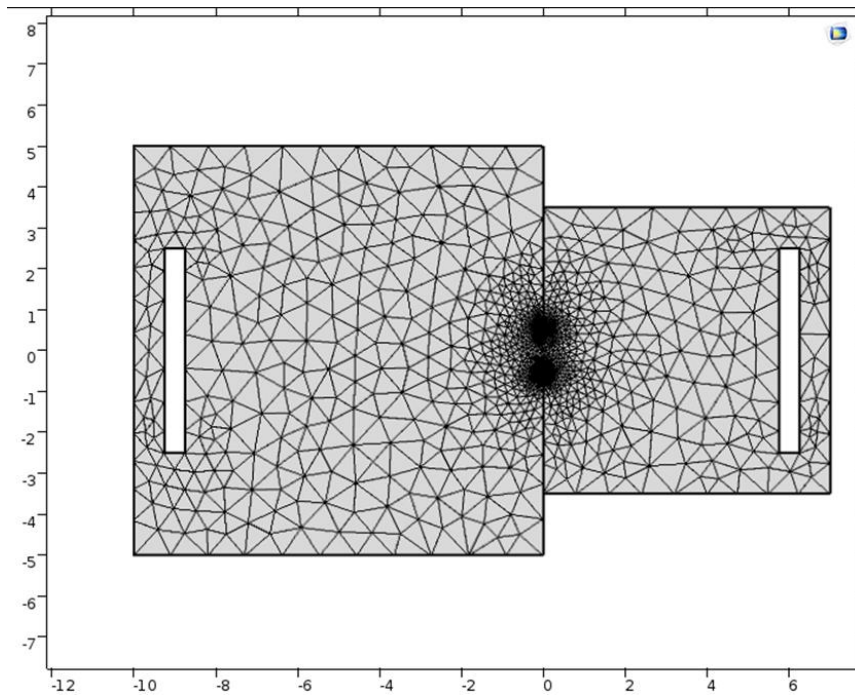


[Figure 3-3] Boundary conditions in chamber

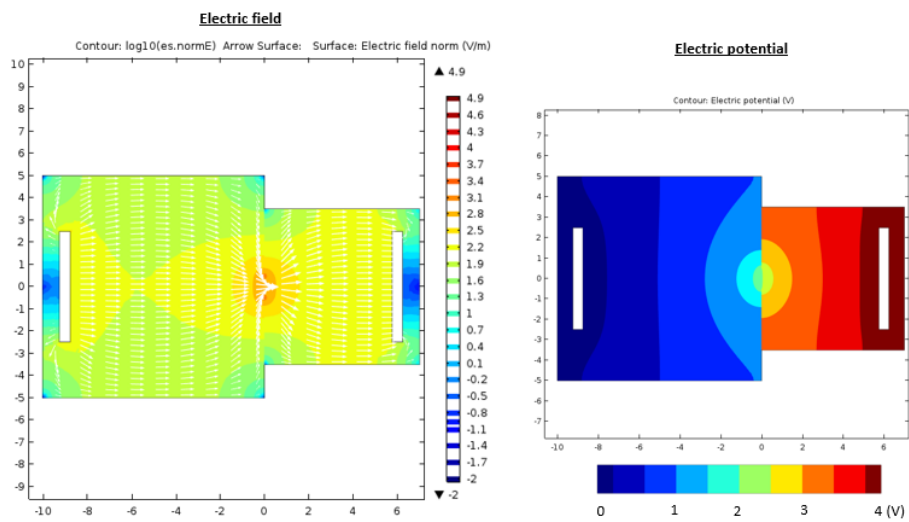
[텍스트 입력]

[텍스트 입력]

[텍스트 입력]



[Figure 3-4] Calculation of mesh



[Figure 3-5] Modeling results

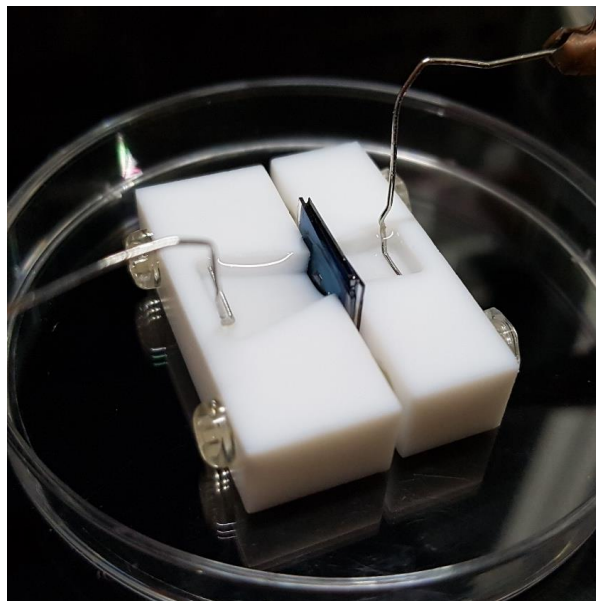
3. Results

Two chamber system was applied to solve the leakage problem. A filter was placed between the reservoir chamber and the collector chamber. [Figure 3-6] To seal between chamber and filter, PDMS was inserted between chamber and filter. After assemble reservoir chamber, PDMS, nanofilter and collector chamber, tighten a screw. [Figure 3-7] The chamber was made of teflon. It is easy to wash and can be used repeatedly. In addition, because DNA does not stick to teflon material, teflon is the material that facilitate DNA experiments. In addition, as previously confirmed, it was shown that the electric field is evenly distributed with respect to the y-axis direction. [Figure 3-7]

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]



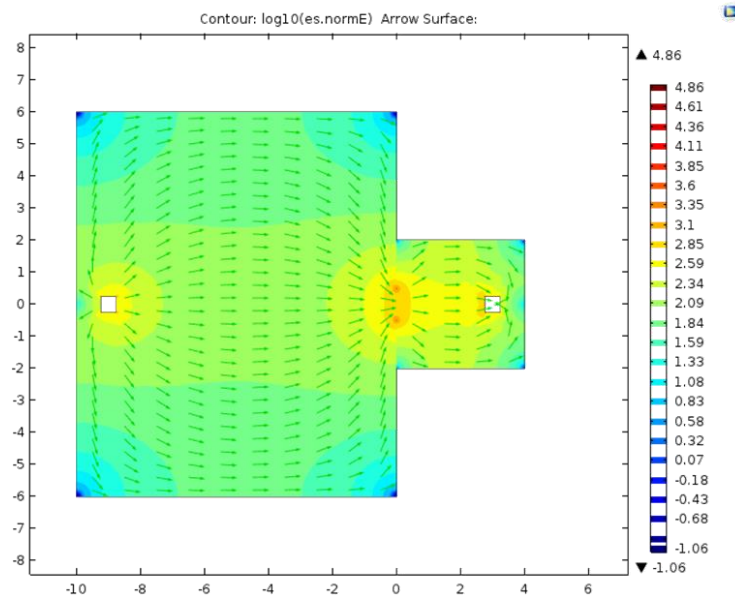
[Figure 3-6] Two chamber system picture

[텍스트 입력]

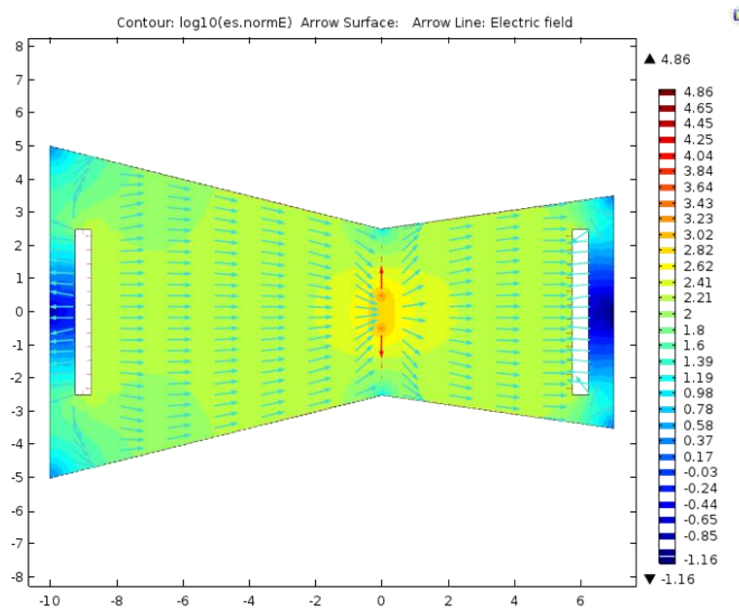
[텍스트 입력]

[텍스트 입력]

Electric field (log scale)



Electric field (log scale)



[Figure 3-7] COMSOL multiphysics simulation result

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]

Chapter 4. Numerical Modeling of DNA Translocation and Experimental Results

1. Introduction

Prior to performing DNA extraction, a pure DNA solution was placed in a reservoir chamber to observe the movement of the DNA. We define this as DNA translocation and conducted DNA translocation experiment according to voltage, time, concentration and various pore sizes. Before conducting the experiment, we derived total DNA translocation amount through numerical modeling in order to predict the pattern of DNA translocation and compare it with actual experimental values.

2. Numerical modeling

The total DNA translocation amount is calculated to show the experimental tendency according to experimental values – time, voltage, concentration of DNA solution and various pore sizes.

When calculating the total DNA translocation amount, we assume three assumptions.

Assumption 1. The flux near membrane determines the overall flux.

Assumption 2. There is no external disturbance in the DNA passing through the pore.

Assumption 3. There is no interaction between DNA molecules.

- Total translocation amount calculation by flux

Total translocation amount could be derived by calculating flux through membrane. Flux is integral of time and effective area. This applies to DNA translocation equation as follows.

$$T_t = \iint J dt \cdot dA$$

Where T_t is total translocation amount, J is DNA flux through membrane, t is time, A is effective area.

- Flux near membrane

In DNA translocation system, concentration of DNA solution near membrane does not change by time. Therefore, flux does not change by time. Also area does not change in system. Thus total translocation amount could be expressed as the product of flux, time and area. The formula is as follows.

$$T_t = \iint J dt \cdot dA = J \cdot t \cdot A$$

And as known, flux is product of concentration and drift velocity. And drift velocity could be calculated as the product of DNA mobility and electric field. Therefore, the flux formula could be expressed as follows.

$$J = c \cdot v = c \cdot \mu \cdot E$$

Where c is concentration of DNA solution, v is drift velocity, μ is DNA mobility and E is electric field in chamber.

-Electric field on membrane

The electric field on membrane is calculated from the applied voltage on membrane. Electric field could be calculated by following equation.

$$E \left(\frac{V}{m} \right) = \frac{V \times \frac{R_{membrane}}{R_{system}}}{T_m}$$

Where E is electric field on membrane, V is applied voltage in system, T_m means thickness of membrane, $R_{membrane}$ represents resistance of membrane, and R_{system} is resistance of system.

-Total translocation amount

So far we have derived the Translocation amount from the flux near the membrane. And this is represented by flux, time, and area. At this time, flux can be obtained by multiplying DNA concentration, DNA mobility and electric field. Taking these results together, we can derive total DNA translocation amount in term of time, area, voltage, DNA mobility and concentration of DNA solution.

$$J = c \times v = c\mu E = c\mu \frac{V \times \frac{R_{membrane}}{R_{system}}}{T_m}$$

$$\begin{aligned} \therefore T_A &= \iint J dt \cdot dA = J \cdot t \cdot A \\ &= c\mu V \frac{A_{chamber}}{L_{chamber}} \frac{1}{N_p} \frac{1}{A_p} t A_p \cdot N_p \end{aligned}$$

$$T_t = c\mu t V \frac{A_{chamber}}{L_{chamber}}$$

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]

There is no term for pore size in this formula. In this equation, there are only variables of concentration of DNA solution, DNA mobility, time, voltage and chamber size. In other words, it can be predicted that the pore size will not affect the DNA translocation amount. Because DNA mobility and chamber size are fixed values, they are not variables that vary from experiment to experiment. In actual DNA translocation experiments, we will look at the trends in translocation amount depending on DNA solution concentration, time, voltage and pore size.

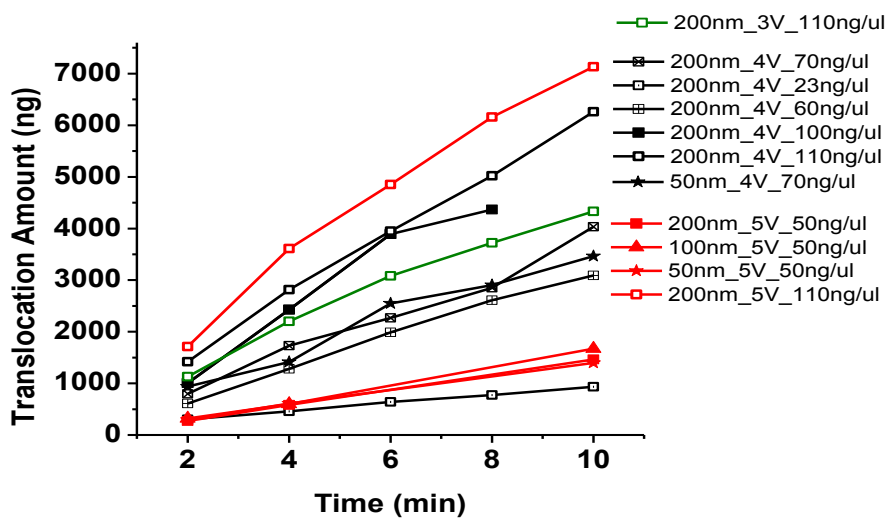
3. Experimental results

Experiment was conducted in various time, voltage, concentration and pore size. [Figure 4-1] All values were measured by NanoDrop 2000C. It measures absorbance in specific wavelengths that represents protein and nucleic acid. Nucleic acid has 260nm wavelengths absorbance where protein is 280nm. By beer's law, the solution concentration was calculated. From concentration, amount was defined. Experiments were performed at different solution concentrations, voltage, time and pore size. In this graph, we can see that the translocation amount increases with time. However, it is difficult to confirm how much it increases. It was also found that when the solution concentration and time were the same, the translocation amount increased as the voltage increased. However, it was also difficult to confirm quantitatively. In order to see the tendency of the experiment clearly, numerical modeling results were compared with actual experiment results. And we will analyze the experimental results according to solution concentrations, voltage, time and pore size.

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]



[Figure 4-1] Experimental results

- 3-1 Time

According to the numerical modeling results, translocation amount should increase proportionally to time. This could be verified by following equation.

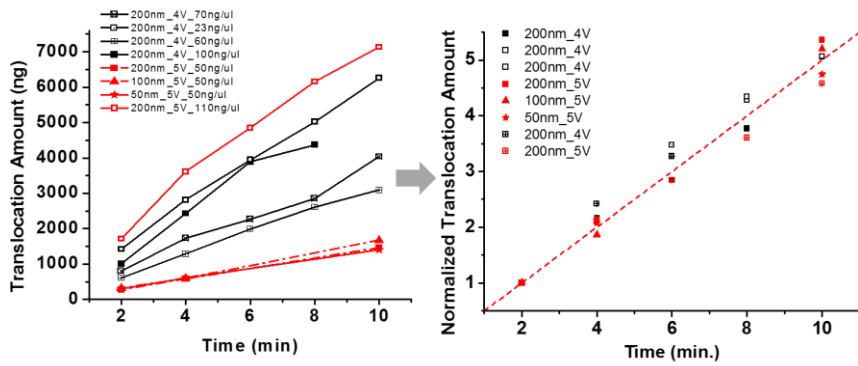
$$\frac{T_t}{T_0} = \frac{c\mu t V \frac{A_{chamber}}{L_{chamber}}}{c\mu t_0 V \frac{A_{chamber}}{L_{chamber}}} = \frac{t}{t_0}$$

The tendency of actual experimental values cannot be seen clearly in the left graph. This is because all experiments were conducted in different conditions. Therefore, to clearly see tendency by time, each translocation experiment results were normalized with each translocation amount in 2 minutes values. The normalized result is shown in right graph. As seen, translocation amount experimental results matches to the modeling results very well. [Figure 4-2]

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]



[Figure 4-2] Experimental results by time

- 3-2 Voltage

According to the numerical modeling results, translocation amount should increase proportionally to voltage. This could be verified by following equation.

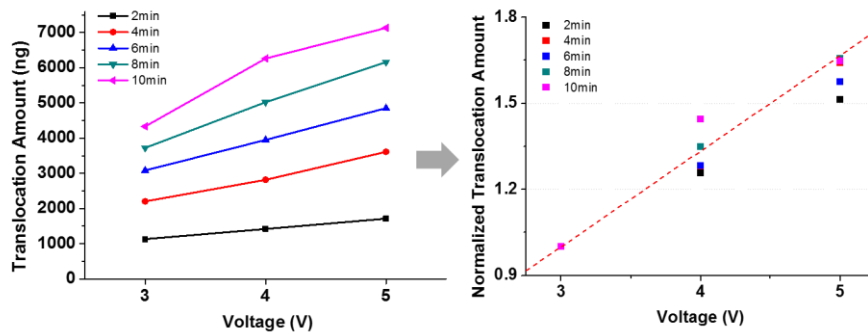
$$\frac{T_t}{T_0} = \frac{c\mu tV \frac{A_{chamber}}{L_{chamber}}}{c\mu tV_0 \frac{A_{chamber}}{L_{chamber}}} = \frac{V}{V_0}$$

The tendency of actual experimental values cannot be seen clearly in the left graph. This is because all experiments were conducted in different conditions. Therefore, to clearly see tendency by voltage, each translocation experiment results were normalized with each translocation amount in 3 voltage values. We conducted translocation experiment over 3V. Under 3V, reaction between electrode and solution cannot be seen so that the potential difference between two electrodes does not exists. We could verify this with small current flow ($\sim 10^{-8}$ A) under 3V. The normalized result is shown in right graph. As seen, translocation amount experimental results matches to numerical modeling equation. [Figure 4-3]

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]



[Figure 4-3] Experimental results by voltage

- 3-3 Concentration

According to the numerical modeling results, translocation amount should increase proportionally to DNA solution concentration. This could be verified by following equation.

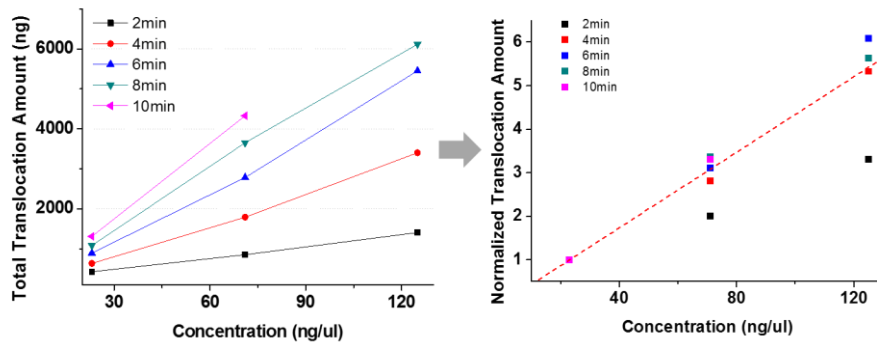
$$\frac{T_t}{T_{c1}} = \frac{c_t c_{\mu} t V \frac{A_{chamber}}{L_{chamber}}}{c_{c1} c_{\mu} t V \frac{A_{chamber}}{L_{chamber}}} = \frac{c_t}{c_{c1}}$$

The tendency of actual experimental values cannot be seen clearly in the left graph. This is because all experiments were conducted in different conditions. Therefore, to see tendency clearly by concentration, each translocation experiment results were normalized. The normalized result is shown in right graph. As seen, translocation amount experimental results matches to numerical modeling equation. But the translocation amount in 2 minutes has quite differences with other results. In 23 ng/ ul concentration and 2 minutes translocation results could be measured inaccurate. The number in nanodrop is too small (~2ng/ul), it reaches the measurement limit. Because of the inaccuracy of the measurement, the reference point – 23ng/ul and 2minutes result – may have over measured or under measured. Therefore, in normalized system, the values over that also could be affected. [Figure 4-4]

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]



[Figure 4-4] Experimental results by concentration

- 3-4 Pore Size

According to the numerical modeling results, translocation amount is irrelevant with pore size in membrane. This could be verified by following equation.

$$T_t = c\mu tV \frac{A_{chamber}}{L_{chamber}}$$

Total translocation amount is product of electric field and effective area.

$$T_t \propto \text{Electric field} \cdot \text{Effective area}$$

Because electric field increases proportionally to resistance of membrane, it decreases proportionally to pore size in membrane. In addition, the bigger the pore size, the bigger the effective area.

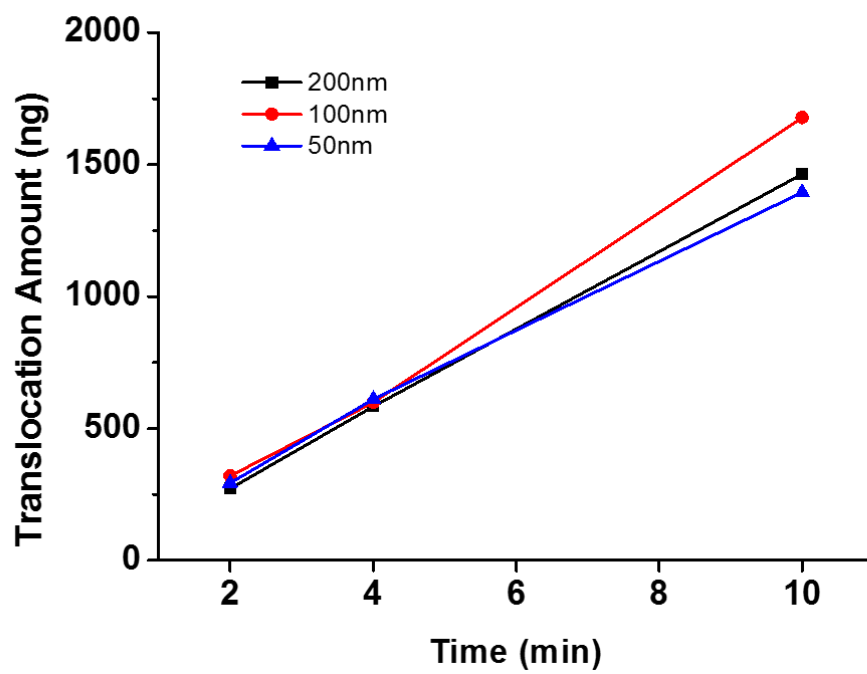
$$\begin{aligned} \text{Electric field} &\propto R_{\text{membrane}} \propto \frac{1}{\text{Pore size}} \\ \text{Effective area} &\propto \text{Pore size} \end{aligned}$$

Translocation amount is product of electric field and effective area, pore size is offset from each other. As a result, translocation amount is regardless of pore size by numerical modeling and experimental results. [Figure 4-5]

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]



[Figure 4-5] Experimental results by pore size

- 3-5 Comparison with real translocation amount and numerical modeling value

Since DNA is moved by the electric field, the DNA translocation amount can be calculated by calculating the electric field on the filter. Compare calculated translocation amount value with experimental values – time, voltage, DNA solution concentration and pore size. All experiments matches with numerical modeling equation. [Figure 4-6]

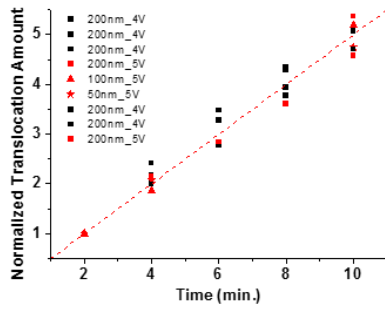
Therefore, the real translocation amount values should be compared with numerical modeling values. The calculated value was found to be 5 times larger than the experimental value. [Figure 4-7] And this could be expressed by following formula.

$$T_t = \alpha c \mu t V \frac{A_{chamber}}{L_{chamber}}, \alpha = 0.20$$

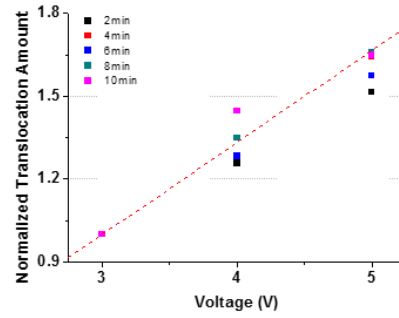
[텍스트 입력]

[텍스트 입력]

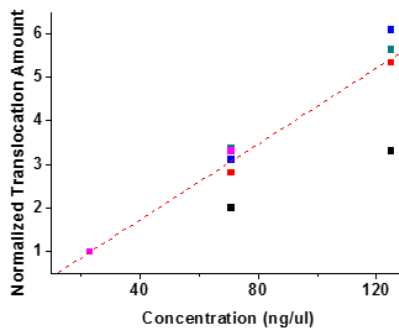
[텍스트 입력]



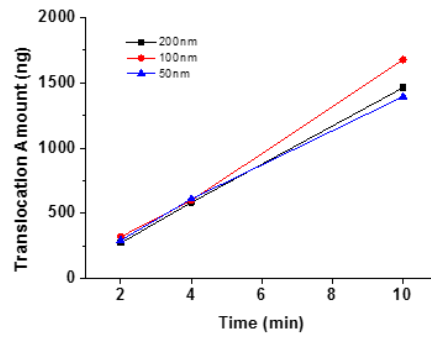
$$T_t \propto t$$



$$T_t \propto V$$



$$T_t \propto c$$



Irrelevant with pore size

[Figure 4-6] Experimental results by variables

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]

	Concentration (ng/ul)	DNA mobility (cm ² /V*s)	Time (s)	Chamber length (cm)	Chamber Area (cm ²)	Applied Voltage (V)	Translocation Amount (ng)	Experimental Concentration (ng/ul)	Experimental Value (ng)	Experimental Value/ Calculation Value
200nm	116.1	0.0004	120	0.7	0.15	4	4776.7	11.4	855	0.18
200nm	116.1	0.0004	240	0.7	0.15	4	9553.4	24.7	1852.5	0.19
200nm	116.1	0.0004	360	0.7	0.15	4	14330.1	32.4	2430	0.17
200nm	116.1	0.0004	480	0.7	0.15	4	19106.7	40.7	3052.5	0.16
200nm	116.1	0.0004	600	0.7	0.15	4	23883.4	53.8	4035	0.17
200nm	125	0.0004	120	0.7	0.15	4	5142.9	20.1	1005	0.20
200nm	71	0.0004	120	0.7	0.15	4	2921.1	12.2	610	0.21
200nm	125	0.0004	240	0.7	0.15	4	10285.7	48.6	2430	0.24
200nm	71	0.0004	240	0.7	0.15	4	5842.3	25.6	1280	0.22
200nm	125	0.0004	360	0.7	0.15	4	15428.6	77.85	3892.5	0.25
50nm	116.1	0.0004	120	0.7	0.15	4	4776.7	13.4	1005	0.21
50nm	116.1	0.0004	240	0.7	0.15	4	9553.4	20.2	1515	0.16
50nm	116.1	0.0004	360	0.7	0.15	4	14330.1	36.4	2730	0.19
50nm	116.1	0.0004	480	0.7	0.15	4	19106.7	41.5	3112.5	0.16
50nm	116.1	0.0004	600	0.7	0.15	4	23883.4	49.5	3712.5	0.16

[Figure 4-7] Comparison with experimental values and numerical modeling values

4. Discussion

The values from numerical modeling and experimental are quite different. (About five times) Several assumptions could cause differences between modeling and real experiment. [Figure 4-8]

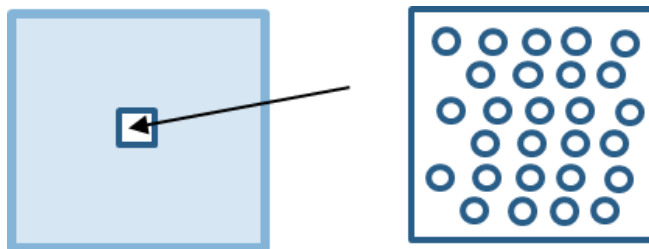
First, in the modeling, pore structure was not considered. According to modeling results, millions pore opening structure and a big size single pore structure regarded as same structure. It means physics near pore does not considered at all. Therefore, modeling and experimental values could be different.

Second, in modeling system, we assume DNA as a molecule. In numerical modeling, a DNA pass through a pore and no interactions between DNAs. However, in real system, DNA should be in solution and could have complex mechanism to pass through membrane.

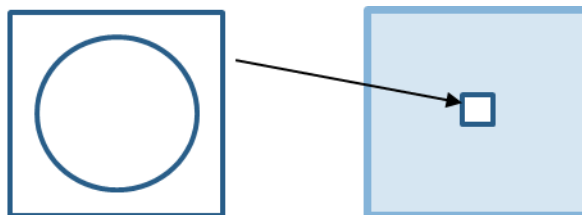
[텍스트 입력]

[텍스트 입력]

[텍스트 입력]



Millions pore in opening area



A pore with same size of that of millions pore

[Figure 4-8] The concept of assumption

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]

Chapter 5. Summary and Conclusion

1. Summary and Conclusion

In the past, the process of extraction and purification of nucleic acids used to be complicated, time-consuming, labor-intensive, and limited in terms of overall throughput. Manual method has certainly come a long way over time with various commercial offerings which included complete kits containing most of the components needed to isolate nucleic acid, but most of them require repeated centrifugation steps, followed by removal of supernatants depending on the type of specimen and additional mechanical treatment. Therefore, in this study, we suggested new extraction system – nanofilter system. The technology should allow a high throughput of samples; the yield, purity, reproducibility, and scalability of the biomolecules as well as the speed, accuracy, and reliability of the assay should be maximal, while minimizing the risk of cross-contamination.

To achieve these requirements, we designed nanofilter. Nanofilter has membrane that is consisted of millions of nano meter size of pores. And during the filter fabrication process or by atomic layer deposition method, pore size was controlled. Nanofilter would block to transfer of protein and cell debris but not nucleic acid. In addition to embody the system, chamber was confirmed by simulating. It confirmed that electric field is evenly distributed through chamber.

To better understand the trends according to the experimental variables, the experimental results were predicted and compared using the numerical model. By the experiment and numerical modeling results, DNA translocation amount increases proportionally with time, voltage, and concentration but irrelevant to pore size. The formula and experimental tendencies matched well. However, the experimental values did not match with modeling results. Some assumptions like –

[텍스트 입력]

[텍스트 입력]

[텍스트 입력]

DNA as a molecule, only one DNA pass through one pore and unconsidered pore structure – may have resulted in this.

Experiments were carried out only on pure lambda DNA, which could be tested in terms of purity improvement through lysis solutions.

Abstract

현재 병의 진단은 생물 분야에서 중요한 이슈이다. 병의 진단은 크게 4 단계로 이루어져 있다. 샘플 채취, 유전 물질 분리, 유전물질 분석을 위한 증폭 그리고 분석이다. 본 연구에서는 기존에 쓰이던 charge 기반 DNA 흡착 키트와 다른 아이디어로 유전물질을 분리하고자 하였다. DNA는 negative charge를 띠는 물질이기 때문에 전기장 안에서 이동할 것이라고 생각하였다. 이 기본적인 원리에서 우리는 DNA를 전기장을 통해 한 쪽 방향으로 이동시키고, 순수 유전물질만을 수집하여 후속 공정인 증폭 공정에 이용하고자 하였다. 전기장 안에서는 charge를 띠는 물질은 모두 이동할 것이다. 하지만 charge to mass ratio가 protein에 비해 큰 DNA가 먼저 그리고 많이 이동할 것이라 예상하여 고 순도의 DNA를 얻을 수 있을 것이다. 이 시스템의 이점은 빠르고 one-step 공정이며 기존에 흡착을 위해 쓰이던 용액들을 쓰지 않아 DNA의 훼손을 최소화 할 수 있는 것에 있다.

본 연구에서는 이 아이디어를 구체화 하기 위해서, filter membrane을 개발 하였다. 이 filter membrane은 50-200nm 크기의 pore가 수백만 개 뚫려 있어, DNA가 이동 하는 데에는 방해가 되지 않고 다른 cell debris들과 protein에게는 방해가 되도록 한다. 본 연구는 앞서 진행된 선행연구가 없는 만큼, 챔버 디자인부터 시행하였다. 전기장의 양상을 시뮬레이션으로 알아보아 챔버 디자인을 확정하였다. 후에는 실험 변수들에 따른 경향성을 잘 알아보기 위해서 실험 결과를 모델을 이용하여 예측하고 비교 하여 해석하였다. 그러나 모델의 한계로 실험값과의 차이가 있었고 그 이유를 논해 보았다.